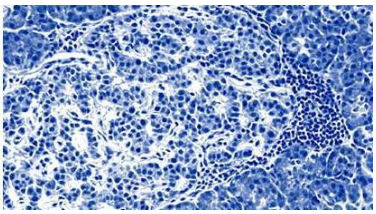
nPOD
Network for Pancreatic Organ Donors with Diabetes



Pharmacologic and Genetic Approaches to Defining Human Beta Cell Mitogenic Targets Of Harmine Family Analogues: Is There More Than DYRK1A?

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PURPOSE

Both Type 1 and Type 2 diabetes result entirely or in part from a reduction in functional pancreatic beta cells, with a resultant loss of adequate insulin secretion. Thus, expansion of functional beta-cells from residual human beta cells in people with Type 1 diabetes (T1D) and Type 2 diabetes (T2D) and regeneration of beta cells in human islets in vitro are attractive approachs for potential diabetic therapies. We and other groups have recently found that small molecule inhibitors of Dual Specificity, Tyrosine Phosphorylation-Regulated Kinase 1A (DYRK1A), such as harmine, INDY, 5-IT and GNF4877, are able to drive human beta cell regeneration in vitro and in vivo. We have also defined the key target relevant to human β-cell replication to be Dual Specificity Tyrosine-Regulated Kinase 1a (DYRK1A). However, Kinome scans and other evidences suggest that there are likely other targets of the "DYRK1A" inhibitors that participate in induction of human beta cell proliferation. While DYRK1A is certainly a target of this class, whether it is the only, or the most important target, is unknown. Here, we try to refine the potential mitogenic targets of the "DYRK1A" inhibitors in human islets.

METHODS

A combination of human beta cell RNAseq, "DYRK1A" inhibitors kinome scans, pharmacologic inhibitors, and adenovirally genetic targeted silencing of candidate genes on human beta cell proliferation was employed to refine the potential mitogenic targets of the harmalog family.

SUMMARY OF RESULTS

We explored the mechanisms of action and targets of the DYRK1A inhibitor class of small molecules on human beta cell proliferation, focusing initially on harmine, INDY, leucettine-41, and subsequently on GNF4877 and 5-IT. Our data confirmed in each case that DYRK1A is the central mitogenic target of the DYRK1A class inhibitor in human islets. Surprisingly, however, DYRK1B but not DYRK2, DYRK3 and DYRK4 also proves to be an important target: silencing DYRK1A leads to a resultant DYRK1B increase; simultaneous silencing of both DYRK1A and DYRK1B yields greater beta cell proliferation than silencing either individually and DYRK1B overexpression effectively blocked proliferation in response to Harmine, 5-IT and GNF4877 to the same degree as DYRK1A overexpression. Importantly, other potential kinases, such as the CLK and the GSK3 families, are excluded as important harmalog targets. Finally, we developed a unique adenovirus tool capable of silencing up to seven targets simultaneously in single cells, which can be useful for studies in human beta cell research.

CONCLUSIONS

Collectively, we report that inhibition of both DYRK1A and DYRK1B is required for induction of maximal rates of human beta cell proliferation, and provide clarity for future efforts at structure-based drug design for human beta cell regenerative drugs.